

CLAIMS

1. A process for the production of egg yolk antibodies binding to small molecule organo chlorine pesticides, the said process comprising the steps of:
 - (a) selecting suitable from poultry birds;
 - (b) immunizing the poultry birds with known complete adjuvant, each ml of said adjuvant comprising heat killed and dried 1 mg of Mycobacterium tuberculosis (H37Ra, ATCC 25177), 0.85 ml paraffin and 0.15 ml mannide monooleate;
 - (c) immunizing the birds with 1000 µg conjugate selected from DDT-OH hapten, Octachloro cyclic hapten, 2,4,5 trichlorophoxyacetic acid β-alanine in breast muscle;
 - (d) immunizing the birds again with the hapten-protein conjugate as given in step (c) with 500 µg of desired hapten conjugate;
 - (e) immunizing the birds with hapten-protein conjugate at the intervals of two, three and five weeks;
 - (f) immunizing the birds thereafter with hapten-protein conjugate at five weeks intervals as long as the bird lays eggs;
 - (g) harvesting antibodies from the egg yolk of the birds.
2. A process as claimed in claim 1, wherein the desired hapten-protein conjugates having binding properties to DDT, Endosulphan and Hexachlorohexane .
3. A process as claimed in claim 1, wherein the production of hapten-protein conjugate, namely DDT-OH binding to DDT, as used in step (c) of claim 1, is as follows:
 - (a) succinylating 2,2,-Bis(4-chlorophenyl) -1,1,1-trichloroethanol overnight, using excess succinic anhydride in pyridine to obtain N-hydroxy succinimide;
 - (b) reacting N-hydroxy succinimide 183.5 mg., 0.5mmol in dichloromethane in the presence of dicyclohexylurea and dimethylaminopyridine catalyst in the ratio 1:1:1:1.2 (hapten:NHS:DCC:DMAP) to convert into N-Hydroxy succinimide active ester; and

- (c) obtaining active ester of DDT-OH hapten for use in conjugation by isolating dicyclohexylurea and evaporating dichloromethane.

4. A process as claimed in claim 1, wherein the production of hapten-protein conjugate namely octachloro cyclic hapten binding to Endosulphan as used in step(c) of claim 1, is as follows:

- (a) dissolving about 3.73 g Heptachlor in 0.1 mol glacial acetic acid by warming;
- (b) dissolving 1.085 g Tert-Butyl hypochlorite, in 0.1 mmol glacial acetic acid and adding to the first solution as obtained in step(a);
- (c) refluxing the mixture on a water-bath for 1 hour;
- (d) separating fine crystals of acetyl-chloro derivative of heptachlor;
- (e) washing the crystals with acetone and drying with air;
- (f) obtaining the crystalline product in a yield of about 3.02 g, m.p. 238 C. 1.09 g
- (g) treating the product to get the pre-hapten 1,3,4,5,6,7,8,8-Octachloro-2-hydroxy-4, 7-methano-3a, and 4,7,7a-tetrahydroindane;
- (h) dissolving the pre hapten in dichloromethane by adding N-hydroxysuccinimide and cooling the mixture to 0°C ;
- (i) adding dicyclohexylcarbodiimide followed by dimethylaminopyridine;
- (j) stirring the mixture overnight; and
- (k) filtering off dichloromethane and evaporating dichloromethane to obtain the active ester of endosulphan.

5. A process as claimed in claim 1, wherein the production of conjugate hapten 2,4,5-Trichloro phenoxy acetic acid β - alanine (TCB) hapten binding to Hexachloro hexane, as per step(c), is as follows:

- (a) adding of β - alanine spacer arm to 2,4,5 Trichlorophenoxyacetic acid by suspending 10mM, 2.55g of 2,4,5 Trichlorophenoxyacetic acid in 5.95 ml thionyl chloride (9.50 mmol);
- (b) refluxing for 1 hour and removing unreacted thionyl chloride by evaporation;

- (c) stirring the product with β - alanine (9 mmol, 0.66g in 7.4 ml of 1M NaOH) at 0°C;
- (d) warming the product for over 16 hours at room temperature;
- (e) isolating the resulting acid by acidification;
- (f) partitioning into ethyl acetate;
- (g) washing with water and brine;
- (h) giving an yield of crude product hapten containing 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) as impurity;
- (i) dissolving the impurity in acetone to obtain colorless flakes of the Trichlorobenzene(TCB) hapten;
- (j) filtering and washing the colourless TCB hapten with acetone and drying in air;
- (k) using silica gel precoated aluminum plates and a mixture of chloroform and methanol in a ratio of 85:15 as eluent showed a single spot in TLC analysis Rf- 0.45 detected by spraying with 2% o-tolidine in acetone and exposure to Uv light/sunlight, at a melting range of 169-70 ° C.
- (l) synthesizing the active ester of hapten 2,4,5-T- β - alanine at melting range of 102-104 °C by dissolving in dichloromethane .
- (m) adding N- hydroxysuccinimide and the mixture is cooled in an ice-bath;
- (n) adding Dimethylsulphoxide(DMSO) dropwise to the mixture until the hapten is dissolved;
- (o) adding Dicyclohexylcarbodiimide to the mixture followed by adding dimethylaminopyridine catalyst;
- (p) stirring the mixture overnight and the temperature slowly raised to the room temperature;
- (q) filtering and evaporating acetone; and
- (r) separating the active ester as a colorless solid.

6. A process as claimed in claim 1, wherein harvesting of antibodies as defined in step(g) of claim 1, is as follows:

- (a) obtaining egg yolk without rupturing the yolk;